(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
8 February 2001 (08.02.2001)

PCT

(10) International Publication Number
WO 01/08714 A1

(51) International Patent Classification[7]: A61L 27/22, 27/36, 27/54, C07K 14/475, 14/51, A61K 48/00

(21) International Application Number: PCT/US00/20630

(22) International Filing Date: 28 July 2000 (28.07.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

09/363,208	28 July 1999 (28.07.1999)	US
09/585,997	2 June 2000 (02.06.2000)	US

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(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
— *With international search report.*
— *Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/08714 A1

(54) Title: CARTILAGE OR BONE MATRIX AS A NUCLEIC ACID DELIVERY VEHICLE

(57) Abstract: The present invention relates to tissues, including but not limited to bone and cartilage implants, as carrier matrices for delivery of biologically active nucleic acids to sites where such tissues are to be implanted. Upon implantation, the nucleic acids associated with the implants are taken up by cells within the implant site and are expressed, to produce growth factors, regenerative factors and the like encoded by the nucleic acids. In this manner, for example, where the nucleic acid encodes bone morphogenetic protein or the like, the rate of bone induction, conduction of healing and the rate of cartilage formation or repair is enhanced, without the attendant need to manufacture, purify, isolate and deliver protein or peptide growth factors.

TITLE OF THE INVENTION

CARTILAGE OR BONE MATRIX AS A NUCLEIC ACID DELIVERY VEHICLE

FIELD OF THE INVENTION

The present invention relates to bone, cartilage and other tissue implant materials as vehicles for delivery of nucleic acid compositions. In specific applications of the invention bone and cartilage implants are treated with nucleic acids actively encoding osteogenic (osteoinductive or osteoconductive) gene products, including but not limited to bone morphogenic proteins, cartilage derived morphogenic proteins, growth factors, and combinations thereof.

BACKGROUND OF THE INVENTION

In the art of orthopedic medicine, there is frequently the need for implantation of materials in order to provide support or to replace damaged or diseased bone or cartilage tissue. Classically, such implants have comprised titanium or other relatively inert metals, synthetic polymeric substances, and the like. Autologous bone, harvested from a first anatomical location, and reimplanted into a second anatomical location, has also been relied upon by surgeons. However, such methods, while effective at the second anatomical location, are less than ideal, due to morbidity at the first anatomical location. Use of allograft (from another individual of the same species) or xenograft (from another species) bone, cartilage or other material has gained increasing acceptance, as techniques for removal of potentially pathogenic organisms have become increasingly sophisticated and reliable.

Spinal fusion is indicated to provide stabilization of the spinal column for painful spinal motion and disorders such as structural deformity, traumatic instability, degenerative instability, and post-resection iatrogenic instability. Fusion, or arthrodesis, is achieved by the formation of an osseous bridge between adjacent motion segments. This can be accomplished within the disc space, anteriorly between contiguous vertebral bodies or

posteriorly between consecutive transverse processes, laminae or other posterior aspects of the vertebrae.

An osseous bridge, or fusion mass, is biologically produced by the body upon skeletal injury. This normal bone healing response is used by surgeons to induce fusion across abnormal spinal segments by recreating spinal injury conditions along the fusion site and then allowing the bone to heal. A successful fusion requires the presence of osteogenic or osteopotential cells, adequate blood supply, sufficient inflammatory response, and appropriate preparation of local bone. This biological environment is typically provided in a surgical setting by decortication, or removal of the outer, cortical bone to expose the vascular, cancellous bone, and the deposition of an adequate quantity of high quality graft material.

A fusion or arthrodesis procedure is often performed to treat an anomaly involving an intervertebral disc. Intervertebral discs, located between the endplates of adjacent vertebrae, stabilize the spine, distribute forces between vertebrae, and cushion vertebral bodies. A normal intervertebral disc includes a semi-gelatinous component, the nucleus pulposus, which is surrounded and confined by an outer, fibrous ring called the annulus fibrosis. In a healthy, undamaged spine, the annulus fibrosis prevents the nucleus pulposus from protruding outside the disc space. Spinal discs may be displaced or damaged due to trauma, disease or aging. Disruption of the annulus fibrosis allows the nucleus pulposus to protrude into the vertebral canal, a condition commonly referred to as a herniated or ruptured disc. The extruded nucleus pulposus may press on the spinal nerve, which may result in nerve damage, pain, numbness, muscle weakness and paralysis. Intervertebral discs may also deteriorate due to the normal aging process or disease. As a disc dehydrates and hardens, the disc space height will be reduced leading to instability of the spine, decreased mobility and pain.

Sometimes the only relief from the symptoms of these conditions is a discectomy, or surgical removal of a portion or all of an intervertebral disc, followed by fusion of the adjacent vertebrae. The removal of the damaged or unhealthy disc will allow the disc

space to collapse. Collapse of the disc space can cause instability of the spine, abnormal joint mechanics, premature development of arthritis or nerve damage, in addition to severe pain. Pain relief via discectomy and arthrodesis requires preservation of the disc space and eventual fusion of the affected motion segments. Bone grafts are often used to fill the intervertebral space to prevent disc space collapse and promote fusion of the adjacent vertebrae across the disc space. In early techniques, bone material was simply disposed between the adjacent vertebrae, typically at the posterior aspect of the vertebrae, and the spinal column was stabilized by way of a plate or rod spanning the affected vertebrae. Once fusion occurred the hardware used to maintain the stability of the segment became superfluous and was a permanent foreign body. Moreover, the surgical procedures necessary to implant a rod or plate to stabilize the level during fusion were frequently lengthy and involved. It was therefore determined that a more optimal solution to the stabilization of an excised disc space is to fuse the vertebrae between their respective end plates, preferably without the need for anterior or posterior plating.

There have been a number of attempts to develop an acceptable intra-discal implant that could be used to replace a damaged disc and maintain the stability of the disc interspace between the adjacent vertebrae, at least until complete arthrodesis is achieved. To be successful the implant must provide temporary support and allow bone ingrowth. Success of the discectomy and fusion procedure requires the development of a contiguous growth of bone to create a solid mass, because the implant may not withstand the cyclic compressive spinal loads for the life of the patient. Many attempts to restore the intervertebral disc space after removal of the disc have relied on metal devices. U.S. Patent No. 4,878,915 to Brantigan teaches a solid metal plug. U.S. Patent Nos. 5,044,104; 5,026,373 and 4,961,740 to Ray; 5,015,247 to Michelson and U.S. Patent No. 4,820,305 to Harms et al., U.S. Patent No. 5,147,402 to Bohler et al. and 5,192,327 to Brantigan teach hollow metal cage structures. Unfortunately, due to the stiffness of the material, some metal implants may stress shield the bone graft, increasing the time required for fusion or causing the bone graft to resorb inside the cage. Subsidence, or sinking of the device into bone, may also occur when metal implants are implanted

between vertebrae if fusion is delayed. Metal devices are also foreign bodies which can never be fully incorporated into the fusion mass.

Various bone grafts and bone graft substitutes have also been used to promote osteogenesis and to avoid the disadvantages of metal implants. Autograft is often preferred because it is osteogenic. Both allograft and autograft are biological materials which are replaced over time with the patient's own bone, via the process of creeping substitution. Unlike a metal implant, over time a bone graft may virtually disappear, while a metal implant persists long after its useful life. Stress shielding is avoided because bone grafts have a similar modulus of elasticity as compared with the surrounding bone. Commonly used implant materials have stiffness values far in excess of both cortical and cancellous bone. Titanium alloy has a stiffness value of 114 Gpa and 316L stainless steel has a stiffness of 193 Gpa. Cortical bone, on the other hand, has a stiffness value of about 17 Gpa. Moreover, bone as an implant also allows excellent postoperative imaging because it does not cause scattering like metallic implants on CT or MRI imaging.

Various implants have been constructed from bone or graft substitute materials to fill the intervertebral space after the removal of the disc. For example, the Cloward dowel is a circular graft made by drilling an allogeneic or autogeneic plug from the illium. Cloward dowels are bicortical, having porous cancellous bone between two cortical surfaces. Such dowels have relatively poor biomechanical properties, in particular a low compressive strength. Therefore, the Cloward dowel is not suitable as an intervertebral spacer without internal fixation due to the risk of collapsing prior to fusion under the intense cyclic loads of the spine. Bone dowels having greater biomechanical properties have been produced and marketed by Regeneration Technologies, Inc., (RTI), 1 Innovation Drive, Alachua, Florida 32615, and have been patented, see U.S. Patent No. 5,814,084. Unicortical dowels from allogeneic femoral or tibial condyles are available. RTI has also developed a diaphysial cortical dowel having superior mechanical properties, which forms the basis of the 5,814,084 patent (the '814 patent). This dowel also provides the further advantage of having a naturally preformed cavity formed by the

existing meduallary canal of the donor long bone. The cavity can be packed with osteogenic materials such as bone or bioceramic.

Unfortunately, the use of bone grafts can present several disadvantages. Autograft is available in only limited quantities. The additional surgery also increases the risk of infection and blood loss and may reduce structural integrity at the donor site. Furthermore, some patients complain that the graft harvesting surgery causes more short-term and long-term pain than the fusion surgery. Allograft material, which is obtained from donors of the same species, is more readily obtained. However, allogeneic bone does not have the osteoinductive potential of autogenous bone and therefore may provide only temporary support. The slow rate of fusion using allografted bone can lead to collapse of the disc space before fusion is accomplished. Both allograft and autograft present additional difficulties. Graft alone may not provide the stability required to withstand spinal loads. Internal fixation can address this problem but presents its own disadvantages such as the need for more complex surgery as well as the disadvantages of metal fixation devices. Also, the surgeon is often required to repeatedly trim the graft material to obtain the correct size to fill and stabilize the disc space. This trial and error approach increases the length of time required for surgery. Furthermore, the graft material usually has a smooth surface which does not provide a good friction fit between the adjacent vertebrae. Slippage of the graft may cause neural and vascular injury, as well as collapse of the disc space. Even where slippage does not occur, micromotion at the graft/fusion-site interface may disrupt the healing process that is required for fusion.

Several attempts have been made to develop a bone graft substitute which avoids the disadvantages of metal implants and bone grafts while capturing advantages of both. For example Unilab, Inc. markets various spinal implants composed of hydroxyapatite and bovine collagen. In each case, developing an implant having the biomechanical properties of metal and the biological properties of bone without the disadvantages of either, has been extremely difficult or impossible.

These disadvantages have led to the investigation of bioactive substances that regulate the complex cascade of cellular events of bone repair. Such substances include bone morphogenetic proteins, for use as alternative or adjunctive graft materials. Bone morphogenetic proteins (BMPs), a class of osteoinductive factors from bone matrix, are capable of inducing bone formation when implanted in a fracture or surgical bone site. Recombinantly produced human bone morphogenetic protein-2 (rhBMP-2) has been demonstrated in several animal models to be effective in regenerating bone in skeletal defects. The use of such proteins has led to a need for appropriate carriers and fusion spacer designs.

Due to the need for safer bone graft materials, bone graft substitutes, such as bioceramics, have recently received considerable attention. The challenge has been to develop a bone graft substitute which avoids the disadvantages of metal implants and bone grafts while capturing the advantages of both. Calcium phosphate ceramics are biocompatible and do not present the infectious or immunological concerns of allograft materials. Ceramics may be prepared in any quantity, which is a great advantage over autograft and even allograft bone graft material. Furthermore, bioceramics are osteoconductive, stimulating osteogenesis in bony sites. Bioceramics provide a porous matrix which further encourages new bone growth. Unfortunately, ceramic implants typically lack the strength to support high spinal loads and therefore require separate fixation before the fusion.

Of the calcium phosphate ceramics, hydroxyapatite(HA) and tricalcium phosphate (TCP) ceramics have been most commonly used for bone grafting. Hydroxyapatite is chemically similar to inorganic bone substance and biocompatible with bone. However, it is slowly degraded. ß-tricalcium phosphate is rapidly degraded in vivo and is too weak to provide support under the cyclic loads of the spine until fusion occurs. Thus, developing an implant having the biomechanical properties of metal and the biological properties of bone without the disadvantages of either has been extremely difficult or impossible.

It recently became apparent that natural bone mineral is not actually as close to the chemistry and structure of hydroxyapatite as was previously believed. (Spector, 21 Clinics in Plastic Surgery 437-444, 1994, the complete text of which is herein incorporated by reference.) Natural bone mineral contains carbonate ions, magnesium, sodium, hydrogenophosphate ions and trace elements. Bone mineral also has a different crystalline structure than HA. Other details of bone chemistry are disclosed in U.S. Patent No. 4,882,149 to Spector. Mimicking the chemistry and microstructure of bone is important to obtain a beneficial modulus of elasticity and resorbption rate.

Several attempts have been made to make materials which are closer to the microstructure of bone. Some disclose removing organic material from bone to yield bone mineral. Some of the materials are used as drug carriers as disclosed in, for example, U.S. Patent No. 5,417,975. U.S. Patent No. 4,882,149 to Spector describes a bone mineral material which is free from fat and bone proteins. The result is a powdery, brittle radiopaque material which can be used to deliver bone growth proteins. The Spector mineral is thought to be closer to natural bone mineral than synthetic calcium phosphate ceramics, but it does not have characteristics which allow it to be shaped into formed objects. U.S. Patent Nos. 4,314,380 to Miyata et al. and 5,573,771 disclose adding collagen or gelatin to bone mineral. However, it is unclear how close these materials are to the natural structure of bone, because the crystalline structure is disrupted when all of the proteins are removed from the treated bone. Urist et al. (110 Arch Surg. 416, 1975) discloses a chemosterilized, antigen-extracted, autodigested, alloimplant which is thought to preserve the morphogenetic potential of the material. McKay, WO98/56433, published 17 December 1998, purported to disclose a bone graft composite and spacers comprising bone stated to have "been processed to remove associated non-collagenous bone proteins", followed by combination through soaking with a bone growth factor. However, that publication neither discloses nor suggests the use of nucleic acids encoding osteogenic gene products. In U.S. Patent No. 5,763,416, matrices were disclosed for treatment with nucleic acids and then contact with bone progenitor cells in a bone progenitor tissue site. That patent neither discloses nor suggests the use of bone or cartilage as the nucleic acid carrier matrix.

In WO97/38729, matrices other than bone impregnated with nucleic acids were disclosed for inducing wound healing. In WO95/22611, as in U.S. Patent No. 5,763,416, nucleic acid treated bone-compatible matrices other than bone or cartilage were disclosed for transfer of nucleic acids to bone implant sites.

In WO99/06563, methods were disclosed for transferring specific nucleic acids in carriers other than bone or cartilage to bony implant sites.

In U.S. Patent No. 5,899,936, a method for removing cells from a harvested tissue, followed by re-population of the tissue with cells of a desired type was disclosed. However, this patent neither discloses nor suggests a bony implant as a matrix for delivery of nucleic acids.

In U.S. Patent No. 5,854,207, combinations of bone morphogenic protein and stimulating factors, optionally in combination with demineralized bone matrix implants was disclosed. However, this patent neither discloses nor suggests the delivery of nucleic acids encoding such proteins in association with a bone matrix for induction of bone or cartilage growth or repair.

Accordingly, this invention, which provides bone or cartilage matrix compositions as a delivery vehicle for nucleic acids, meets a need long felt in the art, as there are substantial disadvantages to use of proteins to induce bone or cartilage repair or formation which are overcome by delivery of nucleic acids encoding such proteins.

SUMMARY OF THE INVENTION

In accordance with one aspect of the invention, bone or cartilage graft compositions, vertebral spacers, and various other bone or cartilagenous implants comprising expressible nucleic acids encoding cartilage or bone growth or repair factors. In one aspect, the invention provides reduced antigenicity bone (RAB) in combination with nucleic acids actively encoding cartilage or bone inducing factors, (osteogenic or chondrogenic factors). In another aspect of this invention, compositions of this invention are prepared and used to replace damaged, diseased or otherwise compromised tissues, either in the spine or in any number of other biological locations.

In another aspect of this invention, shaped or formed chondrogenic or osteogenic (osteoconductive or osteoinductive) compositions are provided wherein demineralized bone or cartilage, mineralized bone or cartilage, or pastes comprising mineralized or demineralized bone and cartilage, are provided as delivery vehicles for osteogenic or chondrogenic expressible nucleic acids. In further aspects of this invention, methods are disclosed for conducting various surgical procedures using the compositions of this invention.

One object of the invention is to provide a bone graft implant having substantially natural mineral structure, reduced antigenicity (reduced immunogenicity), enhanced safety and osteoinductive potential.

Another object of the invention is to provide a cartilage graft implant having substantially natural mineral structure, reduced antigenicity (reduced immunogenicity), enhanced safety and osteoinductive or chondrogenic potential..

Another object of the invention is to provide spacers for engagement between vertebrae which restore the intervertebral disc space and which support the vertebral column while encouraging bone ingrowth and avoiding stress shielding.

Another object of the invention is to provide pins, suture anchors, interference screws, demineralized bone implants, including but not limited to ligaments, oral maxilofacial plates, dowels, posterior lumbar interbody fusion implants, trauma screws and plates, pericardium (for dura, plura, shoulder patch and perioligaments), wedges, chips and pastes comprising bone, cartilage or other tissues, alone or in combination with nucleic acids encoding growth factors, including but not limited to bone morphogenetic proteins, cartilage derived morphogenetic proteins, tissue growth factor (beta1 and the like).

One benefit of the present invention is that it solves many of the problems associated with the use of bone and other graft materials, either from allograft or xenograft materials. The antigen removal process disclosed herein removes immunogenic and potentially disease causing agents while retaining the natural microstructure of bone and other tissues

described herein. This feature allows the use of allograft or xenograft, which is available in virtually unlimited supply. Fortifying the graft with nucleic acids encoding nucleic acids which actively encode bone or cartilage growth factors or osteogenic proteins, makes the graft osteoinductive, thereby making the pain and risk of harvesting autograft unnecessary. An additional benefit is that the invention provides a stable scaffold for bone, cartilage or other tissue ingrowth as the process of fusion or new cartilage or tissue generation occurs.

A further object and another benefit of this invention is that it allows the use of bone grafts without the need for metal cages or internal fixation, due to the increased speed of fusion.

Other objects and further benefits of the present invention will become apparent to persons of ordinary skill in the art from the following written description and accompanying Figures.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a top perspective view of a bone dowel implant according to US Patent No. 5,814,084, treated according to the method of the present invention to include nucleic acids encoding growth factors.

FIG. 2 shows bilateral dowel placement between L5 and the sacrum, using a bone dowel such as that shown in figure 1, including nucleic acids encoding bone growth factors.

FIG. 3 is a perspective view of a cortical bone dowel such as that shown in figure 1, having a chamber and a threaded external feature, and including nucleic acids encoding osteogenic factors.

FIG. 4 is a side perspective view of a bone dowel according to this invention.

FIG. 5 is a cross-section of a bone dowel of this invention.

FIG. 6 is a side elevational view of the bone dowel shown in FIG. 5.

FIG. 7 is a cortical bone ring packed with an osteogenic material including nucleic acids encoding osteogenic factors.

FIG. 8 is a representation of a cortical bone ring embodiment provided by this invention.

FIG. 9 is another embodiment of a cortical bone ring provided by this invention.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

For the purposes of promoting an understanding of the principles of the invention, reference will now be made to the embodiments illustrated in the drawings and specific language will be used to describe the same. It will nevertheless be understood that no limitation of the scope of the invention is thereby intended, such alterations and further modifications in the illustrated spacers, and such further applications of the principles of the invention as illustrated therein being contemplated as would normally occur to one skilled in the art to which the invention relates.

The present invention provides bone and cartilage graft compositions, spacers and surgical procedures. The bone graft compositions include bone or cartilage grafts, in combination with nucleic acids encoding an osteogenic material, such as a bone morphogenic protein (BMP), cartilage derived morphogenic protein, growth factors, peptides (e.g. p15). As is now known in the art, delivery of nucleic acids encoding desirable gene products results in uptake of such nucleic acids and expression of the encoded proteins. The nucleic acids may be so-called naked DNA or RNA, comprising appropriate transcription and translation start and stop signals, as are known in the art.

The nucleic acid may also comprise viral replication signals, and may include recombinant viral vectors encoding nucleic acids or genes, the expression of which is desired in a given implant location.

This invention also provides pins, suture anchors, interference screws, demineralized or mineralized bone implants, including but not limited to ligaments, oral maxilofacial plates, dowels, posterior lumbar interbody fusion implants, trauma screws and plates, pericardium (for dura, plura, shoulder patch and perioligaments), wedges, chips and pastes comprising reduced antigenicity bone, cartilage or other tissues, in combination with nucleic acids encoding growth factors, including but not limited to bone morphogenetic proteins, cartilage derived morphogenetic proteins, tissue growth factor (beta1 and the like). Thus, while emphasis may be placed herein on bone implants for spinal fusions, those skilled in the art will appreciate, based on the instant disclosure, that implants for a wide variety of orthopedic and non-orthopedic applications may benefit by treating such tissues with appropriate nucleic acids encoding osteogenic or chondrogenic factors.

The bone grafts according to this invention may be treated according to the method disclosed herein to remove all of the cellular material, fat and non-collagenous protein that is otherwise associated with bone graft compositions. In preferred embodiments, free collagen is also removed, leaving structural or bound collagen which is associated with bone mineral to form the trabecular struts of bone. Such bone implant material, while depleted of non-collagenous proteins and non-structural collagens and is defatted, still contains the natural crystalline structure of bone. Therefore, such bone graft compositions of this invention have the natural microstructure of bone without the risk of disease transmission or significant immunogenicity or antigenicity. The natural crystalline structure of bone is maintained by the presence of structural collagen in association with the natural bone minerals. This yields a bone graft material with preferred physical and biological characteristics, including the ability to deliver nucleic acids, without attendant immunogenicity or antigenicity.

The presence of structural collagen and the natural mineral structure of bone results in an elasticity and radioopacity which is identical or nearly identical to bone. The material has sufficient resilience and elasticity to retain a formed body and yet remains rigid enough to maintain an open space between bone portions to result in a fusion mass. Demineralized bone matrix or autograft bone treated with nucleic acids may also be employed, but if xenograft bone is to be used, it is preferred that non-collagenous, non-structural proteins are removed.

When bone or cartilage graft materials of this invention are combined with nucleic acids encoding BMPs, CDMPs (cartilage derived morphogenic proteins), other growth factors, the composite is an ideal bone graft substitute. The composite has the natural calcium phosphate structure of bone. This facilitates incorporation and substitution of the graft material, giving the composites a desirable resorbption rate of a few months. This compares favorably to the resorbption rates of known materials which are typically either too fast, slow or unpredictable. For example, allograft typically is resorbed within 12-60 months but may, on the other hand, resorb too quickly before fusion can occur due to an immunogenic response by the patient.

The combination of nucleic acids encoding BMP and other osteogenic factors with the graft material according to this invention provides the osteoinductive potential of autograft without the need for a harvesting surgical procedure at a secondary location, where morbidity may occur. The osteoinductive composites of this invention enhance bone growth into and incorporation of the graft, resulting in fusion more quickly than would occur using bone or cartilage graft material alone. Allograft alone typically requires many months to incorporate and sometimes is never fully incorporated, but is merely encased within the patient's bone. The quicker fusion, occurring within about five months, provided by this invention compensates for the less desirable biomechanical properties of graft and makes the use of internal fixation and metal interbody fusion devices unnecessary. The spacers of this invention are not required to support the cyclic loads of the spine for very long because of the quick fusion rates which reduce the biomechanical demands on the spacer. However, when required, the compositions of this

invention may be used with internal fixation devices or may be reinforced as needed, see WO98/56319, hereby incorporated by reference.

Where the bone graft material of this invention has been treated to remove essentially all non-collagenous proteins and non-structural collagens, the graft may be autogeneic, allogeneic or xenogeneic. The components of bone which could cause disease or prompt the patient's body to reject the graft are removed by the treatment process disclosed herein. Xenogenic bone, such as bovine, ovine, porcine, canine, equine or other bone, is available in virtually unlimited supply. Several osteogenic factors are also available in unlimited supply thanks to recombinant DNA technology. Therefore, the present invention solves all of the problems associated with autograft, allograft and xenograft, including supply, immunogenicity, disease transmission and the need for surgical procedures at secondary sites.

This invention provides the further advantage of exploiting the discovery that bone mineral is an excellent carrier for osteogenic factors, such as nucleic acids encoding BMP's, CDMP's, peptides (e.g. p15) and like factors. Hydroxyapatite, which is similar in chemical composition to the mineral in cortical bone, is an osteogenic factor-binding agent which controls the rate of delivery of certain proteins to the fusion site. Calcium phosphate compositions such as hydroxyapatite are thought to bind bone morphogenic proteins and prevent BMP from prematurely dissipating from the spacer before fusion can occur. It is further believed that retention of the BMP by the agent permits the protein to initiate the transformation of mesenchymal stem cells into bone producing cells or osteoblasts within the device at a rate that is conducive to complete and rapid bone formation and ultimately, fusion across the disc space. The cartilage and bone implant-nucleic acid compositions of this invention have the advantage of including a load bearing member composed of bone or cartilage which naturally binds and provides controlled delivery of nucleic acids encoding osteogenic factors such as bone morphogenic proteins, without at the same time inducing undesirable immune responses in the recipient thereof.

This invention also capitalizes on the discovery that cortical bone, like metal, can be conveniently machined into the various shapes disclosed herein. In some embodiments, the load bearing members define threads on an outer surface. Machined surfaces, such as threads, provide several advantages that were previously only available with metal implants. Threads allow better control of spacer insertion than can be obtained with a smooth surface. This allows the surgeon to more accurately position the spacer, which is extremely important around the critical neurological and vascular structures of the spinal column.

Threads and the like also provide increased surface area which facilitates the process of bone healing and creeping substitution for replacement of the donor bone material and fusion. These features also increase post-operative stability of the spacer by engaging the adjacent vertebral endplates and anchoring the spacer to prevent expulsion. This is a major advantage over smooth grafts. Surface features also stabilize the bone-spacer interface and reduce micromotion to facilitate incorporation and fusion. Methods for producing such external bone features are disclosed in U.S. Patent No. 5,814,084, hereby incorporated by reference.

The graft compositions of this invention can be prepared according to methods disclosed herein. Bone of human or animal source is obtained according to known procedures. The bone is cleaned to remove tissue and blood and is then treated with agents to remove cellular material, fats, noncollagenous proteins, and optionally, to remove non-structural collagens. Typical agents include alcohols and peroxides. In preferred embodiments, the bone material is also treated to remove free collagen, leaving only bound or structural collagen in association with bone minerals. This reduces immunogenicity/antigenicity, without compromising the structural integrity of the bone material. One preferred agent for removing free collagen and associated non-structural antigenic proteins and any remaining fat is a chaotropic agent, such as urea, guanidinium hydrochloride, Triton X-100, Tween, TNBP, or the like, in combination with alcohol and peroxide treatment. The bone graft bone material is then preferably washed with sterile, deionized water and

terminally sterilized by suitable methods, including but not limited to gamma irradiation, vapor-phase peroxide treatment, and the like.

Allograft, autograft, or xenograft bone dowels or other appropriately shaped implants can be packaged fresh frozen or freeze-dried, preferably freeze dried. Sterilization can be provided via aseptic processing or terminally sterilized by ETO, E-beam, or gamma irradiation preferably gamma irradiation. Gamma irradiation allows the procurement and processing of allograft or xenograft under less rigorous environmentally controlled conditions since terminal sterilization offers a significantly higher degree of sterility.

The graft according to this invention may be treated to remove all of the non-collagenous bone proteins leaving a non-immunogenic, disease-free, graft implant material having the natural mineral, microcrystalline structure of bone, with a consistency which retains desired forms. The composition of this invention is preferred because it has a microstructure which is the closest to natural bone of all of the known treated bone products. This bone product also has the radioopacity of natural bone and does not show the dense white image of the bone products of Spector and Geistlich. The product of this invention also provides superior resorbability, particularly when combined with nucleic acid encoding osteogenic factors. Resorbption has been found to advantageously occur within several months as opposed to several years required for the Spector and Geistlich materials or the few weeks of the Urist product. When the material is combined with nucleic acids encoding a bone growth factor, the resorbption time is ample for forming the bony bridge required for fusion and bone healing.

The bone or cartilage materials of this invention are combined with an osteogenic composition or material containing nucleic acids which actively encode a bone growth factor, proteins or peptides. Osteogenic nucleic acids can be applied to the bone material by impregnating the graft with a solution including osteogenic nucleic acid compositions. The allograft, autograft or xenograft is allowed to soak for sufficient time to allow the allograft, autograft or xenograft to absorb the nucleic acid. Additional nucleic acid could be used with the allograft, autograft or xenograft produced according to the method of

this invention by the incorporation of the nucleic acid in a delivery vehicle placed around or in the allograft. In some embodiments, nucleic acid encoding an osteogenic composition can be packed into a chamber defined within a body of the material. In addition, the nucleic acids may be combined with appropriate protein growth factors. The various osteogenic factors, growth factors, proteins, peptides or nucleic acids may be forced into the interstices of the bone or other cartilage implants under vacuum or pressure, or by oscillation between high and low pressure in an appropriate chamber or vessel. The composition may be applied by the surgeon during surgery or the spacer may be supplied with the composition preapplied. In such cases, the osteogenic composition may be stabilized for transport and storage such as by freeze-drying. The stabilized composition can be rehydrated and/or reactivated with a sterile fluid such as saline or water or with body fluids applied before or after implantation. Alternatively, a freeze-dried bone or cartilage matrix may be simply reconstituted in a composition comprising expressible nucleic acids, alone or in combination with protein, peptide or other growth factors, antibiotics, antineoplastics, antiinflammatories or the like. The thus reconstituted implant may then be directly implanted into an appropriate recipient or it may be freeze-dried in combination with the applied and absorbed osteogenic composition. In addition, the thus treated implant may be treated with or cultured with various cell-types to provide a cell-coated matrix incorporating expressible nucleic acids.

The term "osteogenic composition" as used herein means virtually any material that promotes bone growth or healing, including natural, synthetic and recombinant proteins, hormones and the like, cells expressing such factors, and nucleic acids actively encoding such factors. By "actively encoding" is meant the inclusion in a nucleic acid construct of all required signals, including transcriptional promoters and terminators, enhancers, and the like, as known in the art, in order to achieve efficient expression of encoded factors. The osteogenic compositions used in this invention preferably comprise an amount of nucleic acid actively encoding growth factors sufficient to stimulate or induce bone growth or healing of a substantially pure bone inductive factor such as a bone morphogenetic protein in a pharmaceutically acceptable carrier. The preferred osteoinductive factors include, but are not limited to, nucleic acids encoding recombinant

human bone morphogenic proteins (rhBMPs), CDMPs, and nucleic acids encoding such factors. Most preferably, the nucleic acid encodes bone morphogenetic protein rhBMP-2, rhBMP-4 or heterodimers thereof. The concentration of nucleic acid encoding rhBMP-2 or other growth factors (e.g. TGF-β1, TGF-β2, p15, and the like), may be between about 1 ng – 1 mg per gram of implant. Nucleic Acids encoding any bone morphogenetic protein is contemplated including bone morphogenetic proteins designated as BMP-1 through BMP-13, CDMP1 and CDMP2, and various growth factors know in the aft to be beneficial for the induction of bone growth and tissue regeneration. Nucleic acids encoding BMPs are known in the art and may be prepared by one skilled in the art as described in U.S. Patent Nos. 5,187,076 to Wozney et al.; 5,366,875 to Wozney et al.; 4,877,864 to Wang et al.; 5,108,922 to Wang et al.; 5,116,738 to Wang et al.; 5,013,649 to Wang et al.; 5,106,748 to Wozney et al.; and PCT Patent Nos. WO93/00432 to Wozney et al.; WO94/26893 to Celeste et al.; and WO94/26892 to Celeste et al. Hereby incorporated by reference.

In addition, all nucleic acids encoding growth factors, disclosed in U.S. patent No. 5,763,416, stimulating factors as disclosed in U.S. Patent No. 5,854,207, or 5,899,936, in WO97/38729; WO95/22611 or WO99/06563 are also hereby incorporated by reference for use in combination with bone or cartilage matrices as disclosed herein. Furthermore, nucleic acids actively including bone calcification factors, such as in U.S. Patent No. 5,635,374 (hereby incorporated by reference) may also be included in the graft compositions of this invention.

The choice of carrier material for the osteogenic nucleic acid composition is based on the application desired, biocompatibility, biodegradability, and interface properties. The bone growth inducing composition can be introduced into the pores of the bone material in any suitable manner. For example, the composition may be injected into the pores of the graft. In other embodiments, the composition is dripped onto the graft or the graft is soaked in or sprayed with a solution containing an effective amount of the composition to stimulate osteoinduction. Alternatively, the osteogenic composition is infused into the bone under elevated or reduced pressure, or both. All of the method for introducing

proteins as disclosed in U.S. Patent No. 5,854,207 are hereby incorporated by reference as methods for introducing nucleic acids into bone or cartilage matrices. In any event, the pores of the matrix are exposed to the composition for a period of time sufficient to allow the nucleic acid osteogenic composition to thoroughly soak, coat and infuse into the graft. The osteogenic factor, preferably a nucleic acid encoding a BMP, may be provided in freeze-dried form and reconstituted in a pharmaceutically acceptable liquid or gel carrier such as sterile water, physiological saline or any other suitable carrier. The carrier may be any suitable medium capable of delivering the nucleic acids to the graft. Preferably the medium is supplemented with a buffer solution as is known in the art. In one specific embodiment of the invention, nucleic acid encoding rhBMP-2 is suspended or admixed in a carrier, such as water, saline, MFR buffer, liquid collagen or injectable bicalcium phosphate. In a most preferred embodiment, nucleic acid encoding BMP is applied to the pores of the graft and then lypholized or freeze-dried. The nucleic acid graft-BMP composition can then be stored in a sterile container, at room temperature, or at decreased temperature for storage and transport. Alternatively, the osteoinductive nucleic acids can be added at the time of surgery.

Other osteoinductive protein carriers known in the art are available to deliver nucleic acids to a chamber defined within the bone or cartilage material or to locations around the implantation site of the bone material. Potential carriers include calcium sulphates, polylactic acids, polyanhydrides, collagen, calcium phosphates, polymeric acrylic esters, polyphoazines, polyamines, polycarbonates, and the like, and demineralized bone. The carrier may be any suitable carrier capable of delivering the nucleic acids, alone or in combination with proteins, peptides or other biologically active agents. Most preferably, the carrier is capable of being eventually resorbed into the body. One preferred carrier is an absorbable collagen sponge marketed by Integra LifeSciences Corporation under the trade name Helistat® Absorbable Collagen Hemostatic Agent. Another preferred carrier is an open cell polylactic acid polymer (OPLA). Other potential matrices for the compositions may be biodegradable and chemically defined calcium sulfates, calcium phosphates such as tricalcium phosphate (TCP) and hydroxyapatite (HA) and including injectable bicalcium phosphates (BCP), and polyanhydrides. Other potential materials are

biodegradable and are biologically derived, such as bone or dermal collagen. Further matrices are comprised of pure proteins or extracellular matrix components. The osteoinductive material may also be an admixture of nucleic acids encoding BMP and a polymeric acrylic ester carrier, such as polymethylmethacrylate, polyvinylacetate, polyhydroxyethyl methacrylate, and the like. For packing the chambers of the spacers of the present invention, the carriers are preferably provided as a sponge which can be compressed into the chamber or as strips or sheets which may be folded to conform to the chamber. Preferably, the carrier has a width and length which are each slightly greater than the width and length of the chamber. In a preferred embodiment, the carrier is soaked with a nucleic acid encoding rhBMP-2 solution and then compressed into the chamber. The sponge is held within the chamber by the compressive forces provided by the sponge against the wall of the dowel. It may be preferable for the carrier to extend out of the openings of the chamber to facilitate contact of the osteogenic composition with the highly vascularized tissue surrounding the fusion site. The carrier can also be provided in several strips sized to fit within the chamber. The strips can be placed one against another to fill the interior. As with the folded sheet, the strips can be arranged within the spacer in several orientations. Preferably, the osteogenic material, whether provided in a sponge, a single folded sheet or in several overlapping strips, has a length corresponding to the length and width of the chamber.

A preferred carrier is a biphasic calcium phosphate ceramic. Hydroxyapatite/tricalcium phosphate ceramics are preferred because of their desirable bioactive properties and degradation rates in vivo. The preferred ratio of hydroxyapatite to tricalcium phosphate is between about 0:100 and about 65:35. Any size or shape ceramic carrier which will fit into the chambers defined in the load-bearing member are contemplated. Ceramic blocks are commercially available from Sofamor Danek Group, B. P. 4-62180 Rang-du-Fliers, France and Bioland, 132 Route d:Espagne, 31100 Toulouse, France. Of course, rectangular and other suitable shapes are contemplated. The osteoinductive factor is introduced into the carrier in any suitable manner. For example, the carrier may be soaked in a solution containing the factor.

The present invention also provides spacers for maintaining a space between adjacent bones. The spacers include a body composed of bone or cartilage graft in combination with a nucleic acid encoding bone growth factor. The bone source is any suitable bone material preferably of vertebrate origin, including tibial, fibial, humeral, iliac, etc. The graft bodies of this invention include flat spacers, bone dowels, cortical rings, bone chips and any other suitably shaped bone pieces. A preferred body is obtained from the diaphysis of a long bone having a medullary canal which forms a natural chamber in the graft.

In one specific embodiment depicted in Figure 1, the invention provides a spacer 10 for maintaining a space between adjacent bones in a patient. The spacer 10 includes a load-bearing member or body 11 sized and shaped to fit within the space. The body 11 is preferably composed of a natural bone or cartilage material which has optimally been processed to remove associated non-collagenous bone proteins. The bone material contains native collagen materials and naturally associated bone minerals but is substantially free from native non-collagenous protein. Alternatively, demineralized or partially demineralized bone may be used. The chemical composition of the bone material allows it to resiliently retain a shaped body. The shape of the body is preferably formed, and the body machined to have desired surface features, before the bone material is processed according to the methods of this invention. However, in some embodiments a mass of bone is treated as disclosed herein, and then is shaped or machined to form a particular body.

Referring now to Figures 1 and 2, in some embodiments, the body 11 is shaped as a dowel. Dowel shaped bodies are sometimes preferred when the bones are vertebrae to be fused. The dowel 10 includes a wall 12 sized for engagement within the intervertebral space (IVS) to maintain the IVS in proper physiologic orientation. The wall 12 defines an outer engaging surface 13 for contacting the adjacent vertebrae. The wall 12 is preferably cylindrical, so that the bone dowel 10 has a diameter d which is larger than the height h of the IVS between adjacent vertebrae V or the height of the space between the lowest lumbar vertebrae L5 and the sacrum S as depicted in Figure 2.

In another embodiment depicted in Figure 3, the body is a bone dowel 20 which includes a wall 22 having an engagement surface 23. The wall 22 defines a chamber 25 therethrough. Preferably, the load-bearing member is a bone graft obtained from the diaphysis of a long bone having a medullary canal which forms the chamber 25. Such dowels are available from Regeneration Technologies, Inc., 1 Innovation Drive, Alachua, Florida 32615. The chamber 25 can be packed with an osteogenic composition comprising nucleic acids encoding BMP, CDMP and the like to stimulate osteoinduction. The chamber 25 is preferably defined through a pair of outer engaging surfaces 23 so that the composition has maximum contact with the endplates of the adjacent vertebrae. Referring now to FIG. 4, the spacer 20 preferably includes a solid protective wall 26 which is positionable to protect the spinal cord from escape or leakage of material packed within the chamber 25. In anterior approaches, the protective wall 26 is posterior. Preferably, the osteogenic composition has a length which is greater than the length of the chamber (Figures 5 and 6) and the composition is disposed within the chamber 25 to contact the end plates of adjacent vertebrae when the spacer 20 is implanted between the vertebrae. This provides better contact of the composition with the end plates to stimulate osteoinduction.

Various features can be machined on the outer surfaces of the dowels of this invention. In one embodiment shown in Figure 3, the dowel 20 includes an outer engaging surface 23 defining threads 24. Referring again to Figure 1, in some embodiments, the dowel 10 is provided with a tool-engaging hole 19 in a wall 18 opposite the solid protective wall 16. The tool engaging hole 19 is provided in a surface of the dowel which is adjacent the surgeon and opposite the initial thread 17. For an anterior procedure, the tool engaging tool hole 19 would be provided in the anterior surface of the dowel 10. Other machined features are contemplated in the outer or bone engaging surfaces 23. Such machine features include surface roughenings such as knurlings and ratchetings.

The spacers of this invention can be inserted using conventional techniques and known tools. In accordance with additional aspects of the present invention, methods for implanting an interbody fusion spacer, such as the spacer 20, are contemplated. The

spacers of this invention can also be inserted using laporoscopic technology as described in Sofamor Danek USA's Laproscooic Bone Dowel Surgical Technique, 1995, 1800 Pyramid Place, Memphis, Tennessee 38132,1-800-933-2635. Devices of this invention can be conveniently incorporated into Sofamor Danek's laproscopic bone dowel system that facilitates anterior interbody fusions with an approach that is much less surgically morbid than the standard open anterior retroperitoneal approaches. This system includes templates, trephines, dilators, reamers, ports and other devices required for laproscopic dowel insertion.

The body may also include other shapes such as cortical rings as shown in Figure 7. Such cortical rings 50 are obtained by a cross-sectional slice of the diaphysis of a long bone and include a superior surface 51 and an inferior surface 52. The graft shown in Figure 7 includes an outer surface 53 which is adjacent and between the superior 51 and inferior 52 surfaces. In one embodiment bone growth through-holes 53a are defined through the outer surface 53 to facilitate fusion. The holes 53a allow mesenchymal stem cells to creep in and bone growth protein and nucleic acids encoding such proteins to diffuse out of the graft. This facilitates bone graft incorporation and possibly accelerates fusion by forming anterior and lateral bone bridging outside and through the device. In another embodiment the outer surface 53 defines a tool-engaging hole 54 for receiving an implanting tool. In a preferred embodiment, at least one of the superior and/or inferior surfaces 51, 52 are roughened for gripping the end plates of the adjacent vertebrae. The surface roughenings may include teeth 56 on ring 50' as shown in Figure 8 or waffle pattern 57 as shown on ring 5O" in Figure 9. When cortical rings are used as the graft material the ring 50 may be trimmed for a more uniform geometry as shown in Figure 7 or left in place as shown in Figure 9.

The graft can also be formed into a square shape to be conveniently incorporated into current surgical procedures such as, the Smith-Robinson technique for cervical fusion (Smith, M.D., G.W. and R.A. Robinson, M.D., "The Treatment of Certain Cervical-Spine Disorders By Anterior Removal Of The Intervertebral Disc And Interbody Fusion", J. Bone And Joint Surgery, 40-A:607-624 (1958) and Cloward, M.D., R.B., "The Anterior

Approach For Removal Of Ruptured Cervical Disks", in meeting of the Harvey Cushing Society, Washington, D.C., April 22, 1958). In such procedures, the surgeon prepares the endplates of the adjacent vertebral bodies to accept a graft after the disc has been removed. The endplates are generally prepared to be parallel surfaces with a high-speed burr. The surgeon then typically sculpts the graft to fit tightly between the bone surfaces so that the graft is held by compression between the vertebral bodies. The bone graft is intended to provide structural support and promote bone ingrowth to achieve a solid fusion of the affected joint. The spacers of this invention avoid the need for this graft sculpting as spacers of known size and dimensions are provided. This invention also avoids the need for a donor surgery because the osteoinductive properties of autograft are provided by the allograft or xenograft implants prepared according to the present invention. The spacers are combined with osteoinductive materials including but not limited to nucleic acids actively encoding growth factors that make allograft or xenograft implants osteoinductive. Therefore, the spacers of this invention speed the patient's recovery by reducing surgical time, avoiding a painful donor surgery and inducing quicker fusion. The following specific examples are provided for purposes of illustrating the invention, and no limitations on the invention are intended thereby.

EXAMPLE 1

REMOVAL OF ANTIGENS FROM BONE GRAFT MATERIAL

This procedure, and variations on the specifics thereof, is employed to remove non-collagenous protein from bone graft materials. The bone graft material may be allograft or xenograft, selected from bovine, porcine, equine, ovine, canine or the like. This procedure removes proteins, fats, polysaccharides, glycosaminoglycans and other non-collagenous antigens from bone matrix, and may be conducted in any order of steps, although carrying the process out in the order provided herein has provided consistently excellent results. For purposes of this invention, it is not critical to carry out this treatment if autograft or allograft bone or cartilage material is used as the nucleic acid delivery matrix.

1. Peroxide Treatment:

This procedure is followed to de-fat the bone tissue, to remove blood and other proteins, and to inactivate microorganisms that might be present in or on the bone. Prior to initiating this treatment, the bone was cleaned of any attached adventitious tissue.

a. The bone tissue was placed into a container, covered with peroxide solution, and permitted to soak with agitation, sonication or both for about 15 minutes.

b. The peroxide solution and removed debris was decanted, and the bone tissue was rinsed with warm sterile water.

Treatment at this stage with a TNBP/Triton X-100 or like solutions, such as hydrogen peroxide/SDS helps to remove additional non-structural proteins and residual fat.

2. Acetone Treatment:

This procedure was followed to remove residual fatty tissue:

a. The bone tissue was placed into a container, covered with acetone, and heated to between about 35 to 40 degrees centigrade, and permitted to soak with agitation for about 15 minutes. This step was repeated until no fat was visible in the solution after being allowed to cool (three to five cycles is usually adequate).

b. The bone tissue was then rinsed with sterile water and permitted to dry.

Variations on this treatment may include use of 99% isopropanol, hexane, and combinations of these solvents. Treatment of the graft at this or a different stage of the process with acetic or other acid (acetic acid, hydrochloric acid, hydrofluoric acid, phosphoric acid, citric acid, formic acid, butyric acid, or mixtures thereof), is useful to produce a slightly demineralized bone graft of reduced antigenicity, with concomitant effects on the graft strength, growth factor binding capacity, resorbability, removal of

acid soluble proteins and loosely associated collagens, and further reductions in antigenicity. We have discovered that reduction in the mineral content of between about 0 to about 25%, or between about 1 to about 10% or even as little as 1% to 5% as compared to the normal bone mineral content confers significant advantages on the reduced antigenicity bone composition. The guiding principle in the level of demineralization that should be conducted is to remove as much mineral as possible, without at the same time reducing the compressive strength of the bone. In order to achieve uniform, limited demineralization, the graft is preferably contacted for about thirty minutes with acid, e.g. 1% acetic acid, with the acid being introduced into an evacuated chamber containing the graft, such that uniform acid penetration occurs. If inorganic acids are used, e.g. HCl, the acid strength or period of acid contact should be reduced, to avoid complete demineralization of the graft. We have found that limited removal of even as little as 1-2% of the normal bone mineral content results in greater predictability (reduced scatter in shear stress measurments) in the strength of bone grafts thus treated. Additional benefits of this treatment include dissolution of acid soluble proteins, efficient removal of SDS or other ionic solvents or contaminants, enhanced binding of growth factors, reduced time to remodel implanted bone, and further reduction in antigenicity.

3. Urea Treatment:

This procedure was followed in order to remove associated non-collagenous proteins from the bone tissue.

a. The tissue was transferred to a container sufficient to contain the tissue and a large excess volume (approximately five-fold) of urea solution (6 M).

b. Non-collagenous proteins were extracted from the bone tissue for approximately 48 hours, with agitation.

c. The urea/protein solution was then decanted, and the bone tissue was rinsed with sterile water, several times(about three) using at least a two-fold volume of water. Each rinse was permitted to continue with agitation for about 20 minutes.

d. A final water wash was conducted for 24 hours with agitation, followed by decantation of the water and freeze-drying of the tissue.

The foregoing procedure was conducted with bovine bone blocks and cancellous chips. Bone cubes of 1 cm were cut from bovine condyles. As a final sterilization step, the thus treated bone was subjected to lyophilization and then gamma irradiation. Bone implant material treated according to this procedure was implanted into a primate model. Little or no adverse immune response (swelling, inflammation) was detected. Furthermore, bone implant treated in this manner was soaked with bone morphogenic protein and implanted in a primate model. Excellent induction of new bone growth into and around the implant bovine bone was detected, without adverse immune response. Based on the success achieved in using bone implant prepared as described herein for delivery of growth factors in the form of active protein, success in delivering cells expressing growth factors, or nucleic acids actively encoding growth factors is expected. Allograft or xenograft bone infused or coated with cells, recombinant or natural, which produce growth factors, or nucleic acid constructs, such as those disclosed in US Patent No. 5,763,416, hereby incorporated by reference, or WO 99/06563, also hereby incorporated, are anticipated to actively induced bone growth without induction of adverse immune responses.

Alternatives to the above-described treatment includes the use of guanidinium hydrochloride, TritonX-100, Tween, TNBP and the like, optionally including combinations of chaotropic agents and surfactants such as SDS (sodium dodecyl sulfate). Examples of conditions for use of these agents include use of 4 M guanidinium hydrochloride, and 1% TNBP/TritonX-100.

EXAMPLE 2

PREPARATION OF DIAPHYSIAL CORTICAL BONE DOWEL

A consenting donor (i.e., donor card or other form of acceptance to serve as a donor) was screened for a wide variety of communicable diseases and pathogens, including human immunodeficiency virus, cytomegalovirus, hepatitis B, hepatitis c and several other pathogens. These tests may be conducted by any of a number of means conventional in the art, including but not limited to ELISA assays, PCR assays, or hemagglutination. Such testing follows the requirements of: (i) American Association of Tissue Banks, Technical Manual for Tissue Banking, Technical Manual - Musculoskeletal Tissues, pages M19-M20; (ii) The Food and Drug Administration, Interim Rule, Federal Register/Vol. 50, No. 238/Tuesday, December 14, 1993/Rules and Regulations/65517, D. Infectious Disease Testing and Donor Screening; (iii) MMWR/Vol. 43/No. RR-8, Guidelines for Preventing Transmission of Human Immunodeficiency Virus Through Transplantation of Human Tissue and Organs, pages 4-7; (iv) Florida Administrative Weekly, Vol. 10, No. 34, August 21, 1992, 59A-l.OOl-O14 59A-l.005(12)(c), F.A.C., (12)(a)-(h), 59A-l.005(15), F.A.C., (4)(a)-(8). In addition to a battery of standard biochemical assays, the donor, or their next of kin, was interviewed to ascertain whether the donor engaged in any of a number of high risk behaviors such as having multiple sexual partners, suffering from hemophilia, engaging in intravenous drug use etc. After the donor was ascertained to be acceptable, the bones useful for obtention of the dowels were recovered and cleaned.

A dowel was obtained as a transverse plug from the diaphysis of a long bone using a diamond tipped cutting bit which was water cleaned and cooled. The bit was commercially available (Starlite, Inc) and had a generally circular nature and an internal vacant diameter between about 10 mm to about 20 mm. The machine for obtention of endo- and cortical dowels consisted of a pneumatic driven miniature lathe which is fabricated from stainless steel and anodized aluminum. It has a spring-loaded carriage which travels parallel to the cutter. The carriage rides on two runners which are 1.0 inch stainless rods and has a travel distance of approximately 8.0 inches. One runner has set

pin holes on the running rod which will stop the carriage from moving when the set pin is placed into the desired hole. The carriage is moveable from side to side with a knob which has graduations in metric and in English. This allows the graft to be positioned. On this carriage is a vice which clamps the graft and holds it in place while the dowel is being cut. The vice has a cut out area in the jaws to allow clearance for the cutter. The lathe has a drive system which is a pneumatic motor with a valve controller which allows a desired RPM to be set.

First, the carriage is manually pulled back and locked in place with a set pin. Second, the graft is loaded into the vice and is aligned with the cutter. Third, the machine is started and the RPM is set, by using a knob on the valve control. Fourth, the set pin, which allows the graft to be loaded onto the cutter to cut the dowel. Once the cutter has cut all the way through the graft the carriage will stop on a set pin. Fifth, sterile water is used to eject dowel out of the cutter. It is fully autoclavable and has a stainless steel vice and/or clamping fixture to hold grafts for cutting dowels. The graft can be positioned to within 0.001" of an inch which creates dowel uniformity during the cutting process.

The cutter used in conjunction with the above machine can produce dowels ranging from 5 mm to 30 mm diameters and the sizes of the cutters are 10.6 mm; 11.0 mm; 12.0 mm; 13.0 mm; 14.0 mm; 16.0 mm; and 18.0 mm. The composition of the cutters is stainless steel with a diamond powder-cutting surface which produces a very smooth surface on the wall of the dowels. In addition, sterile water is used to cool and remove debris from graft and/or dowel as the dowel is being cut (hydro infusion). The water travels down through the center of the cutter to irrigate as well as clean the dowel under pressure. In addition, the water aids in ejecting the dowel from the cutter.

The marrow was then removed from the medullary canal of the dowel and the cavity cleaned to create a chamber. The chamber interior may be scraped or machined as desired and may be filled with desired osteogenic materials, including allograft, autograft, ceramic, growth factors and the like. The final machined product may be stored, frozen or freeze-dried and vacuum-sealed for later use. In an embodiment of this invention, the

dowel is constructed with a nucleic acid encoding BMP. In another embodiment, the dowel is constructed with a mixture of nucleic acids encoding a variety of different growth factors.

EXAMPLE 3
THREADING OF DOWELS

A diaphysial cortical bone dowel is prepared as described above. The plug is then machined, preferably in a class 10 clean room, to the dimensions desired. The machining is preferably conducted on a lathe such as a jeweler's lathe or machining tools may be specifically designed and adapted for this purpose. A hole is then drilled through the anterior wall of the dowel. The hole is then tapped to receive a threaded insertion tool. The thus-prepared dowel is treated with a nucleic acid as described in Example 2.

EXAMPLE 4
PREPARATION OF BONE DOWEL-rhBMP-2 NUCLEIC ACID COMPOSITE BY DRIPPING

A threaded bone dowel is obtained through the methods described above. A vial containing 4.0 mg of lypholized nucleic acid encoding rhBMP-2 is constituted with 1 mL sterile water (Abbott Laboratories) for injection to obtain a 4.0 mg/mL solution as follows:

1. Using a 3-cc syringe and 22G needle, slowly inject 1.0 mL sterile water for injection into the vial containing lypholized rhBMP-2 encoding nucleic acid.
2. Gently swirl the vial until a clear solution is obtained. Do not shake.

The dilution scheme below is followed to obtain the appropriate rhBMP-2 nucleic acid concentration. This dilution provides sufficient volume for two dowels. The dilutions are performed as follows:

1. Using a 5-cc syringe, transfer 4.0 mL of MFR 906 buffer (Genetics Institute) into a sterile vial.
2. Using a l-cc syringe, transfer 0.70 mL reconstituted rhBMP-2 nucleic acid into the vial containing the buffer.
3. Gently swirl to mix.

DILUTION SCHEME

INITIAL rhBMP-2 NUCLEIC ACID CONCENTRATION (mg/mL)	rhBMP-2 Nucleic Acid VOLUME (mL)	MFR-842 VOLUME (mL)	FINAL rhBMP-2 NUCLEIC ACID CONCENTRATION (mg/mL)
4.0	0.7	4.0	0.6

1. Using a 3-cc syringe and 22G needle, slowly drip 2.0 mL of 0.60 mg/mL rhBMP-2 nucleic acid solution onto the Bone Dowel.
2. Implant immediately.

As an alternative to the above, a bone implant is lyophilized, and then brought into contact with a solution containing nucleic acids encoding osteogenic proteins, growth factors, in order to reconstitute the lyophilized bone dowel. In the process of being reconstituted, the dowel draws the nucleic acids encoding growth factors or other osteogenic compositions, natural or recombinant, into the interstices of the bone matrix. The composition of nucleic acid may also include proteins, peptides or other biologically active constituents.

EXAMPLE 5

PREPARAION OF BONE ALLOGRAFT OR XENOGRAFT BONE-BMP NUCLEIC ACID COMPOSITE BY SOAKING

1. Freeze dried nucleic acid encoding rhBMP-2 is reconstituted with sterile water for injection as in Example 4.

2. A sterile allograft or xenograft bone dowel is transferred to a sterile "soaking" container.

3. Reconstituted nucleic acid encoding rhBMP-2 is added to the soaking container so that the allograft is completely submersed in the solution.

4. The allograft or xenograft bone dowel is allowed to soak in the rhBMP-2 encoding nucleic acid solution for 30-60 minutes so that the graft absorbs the nucleic acid, and any other components added to the solution.

To enhance the efficiency of loading of nucleic acids encoding BMPs or other growth factors, autograft, allograft or xenograft is contacted with nucleic acids encoding such factors under vacuum with swirling for about 15 minutes.

EXAMPLE 6

BONE DOWEL PACKED WITH NUCLEIC ACID ENCODING BMP-2/COLLAGEN COMPOSITION

A threaded dowel is obtained through the methods of Examples 1-5. A vial containing 4 .0 mg of lypholized nucleic acid encoding rhBMP-2 is constituted with 1 mL sterile water or saline solution for injection to obtain a 4.0 mg/mL solution as follows:

1. Using a 3-cc syringe and 22G needle, slowly inject 1.0 mL sterile water or saline solution for injection into the vial containing lypholized nucleic acid encoding rhBMP-2.

2. Gently swirl the vial until a clear solution is obtained. Do not shake. The dilution scheme below is followed to obtain the appropriate nucleic acid concentration. The dilutions are performed as follows:

1. Using a 3-cc syringe, transfer 2.5 mL of MFR-842 buffer (Genetics Institute) into a sterile vial.

2. Using a 1-cc syringe, transfer 0.30 mL of 4.0 mg/mL reconstituted nucleic acid encoding rhBMP-2 into the vial containing the buffer.

3. Gently swirl to mix.

DILUTION SCHEME

INITIAL rhBMP-2 NUCLEIC ACID CONCENTRATION (mg/mL)	rhBMP-2 Nucleic Acid VOLUME (mL)	MFR-842 VOLUME (mL)	FINAL rhBMP-2 NUCLEIC ACID CONCENTRATION (mg/mL)
4.0	0 .3	2.5	0 .43

The rhBMP-2 nucleic acid solution is applied to a Helistat sponge (Genetics Institute) as follows:

1. Using sterile forceps and scissors, cut a 7.5 cm x 2.0 cm strip of Helistat off of a 7.5 x 10 cm (3" x 4") sponge.

2. Using a 1-cc syringe with a 22-G needle, slowly drip approximately 0.8 mL of 0.43 mg/mL rhBMP-2 nucleic acid solution uniformly onto the Helistat sheet.

3. Using sterile forceps, loosely pack the sponge into the chamber of the dowel.

4. Using a 1-cc syringe with a 22-G needle, inject the remaining 0.8 mL of 0.43 mg/mL rhBMP-2 nucleic acid into the sponge in the dowel through the openings of the chamber.

5. Implant immediately.

EXAMPLE 7

DOWEL PACKED NUCLEIC ACID ENCODING rhBMP-2/HA/TCP COMPOSITION

A threaded dowel is obtained through the methods of Examples 1-5. A vial containing 4.0 mg of lypholized nucleic acid encoding rhBMP-2 is constituted with 1 mL sterile water or saline solution for injection to obtain a 4.0 mg/mL solution as follows:

1. Using a 3-cc syringe and 22G needle, slowly inject 1.0 mL sterile water for injection into the vial containing lypholized rhBMP-2 nucleic acid.

2. Gently swirl the vial until a clear solution is obtained. Do not shake. A cylindrical block of biphasic hydroxyapatite/tricalcium phosphate (Bioland) is wetted with a 0.4 mg/mL rhBMP-2 nucleic acid solution. The nucleic acid-ceramic block is packed into the chamber of the dowel and the thus packed dowel is then implanted.

EXAMPLE 8

ALLOGRAFT OR XENOGRAFT BONE CHIP-NUCLEIC ACID COMPOSITE PREPARATION

1. Allograft or xenograft bone chips are harvested, processed and prepared according to Example 1 to produce allograft or xenograft bone chips.

2. Freeze dried nucleic acid encoding rhBMP-2 is reconstituted with sterile water or saline for injection as described in Example 4.

3. The sterile allograft or xenograft bone chips are transferred to the sterile "soaking" container. Preferably, the bone chips are first lyophilized, so that upon contact with the BMP nucleic acid solution, the bone chips reconstitute, thereby soaking up the BMP nucleic acid solution into the interstices of the chips.

4. Reconstituted rhBMP-2 nucleic acid is placed into the soaking container so that the allograft or xenograft is completely submersed.

5. The allograft or xenograft bone chips are soaked in the rhBMP-2 nucleic acid solution for 30-60 minutes.

6. Using sterile forceps, the allograft or xenograft bone chips are removed from the soaking container and placed into the posterolateral gutters of the level of the spine to be fused, or into any other bony location where bone fusion or repair is desired.

This procedure may be employed to make a gelatin nucleic acid sponge by injecting gelatin into bone chips prepared as described above and then lyophilizing the composition. As with Example 5, the efficiency of loading of BMP or other growth

factor encoding nucleic acids is enhanced when contact is made with the graft under vacuum.

EXAMPLE 9

PREPARATION OF CORTICAL RING-COMPOSITES

A cortical ring is obtained as a cross-sectional slice of the diaphysis of a human long bone and then optionally prepared using the methods described in Example 1 to produce a cortical ring of reduced antigenicity. The cortical ring is fashioned into a square hollow ring. The ring is packed with an osteogenic composition comprising nucleic acids actively encoding growth factors as described in the foregoing EXAMPLES.

EXAMPLE 10

SPACERS

A D-shaped cervical spacer is obtained as a cross-sectional slice of a diaphysis of a long bone and treated according to the method of Example 1. The exterior surfaces of the walls are formed by machining the slice to a D-shape. The engaging surfaces of the spacer are provided with knurlings by a standard milling machine. A hole is then drilled through the anterior wall of the spacer. The hole is then tapped to engage a threaded insertion tool. The chamber of the spacer is then packed with an osteogenic nucleic acid composition as described in the foregoing EXAMPLES.

EXAMPLE 11

ANTEROR INTERBODY CERVICAL FUSION

The cervical spine is approached anteriorly according to known surgical techniques. The composite material of this invention including osteogenic factor encoding nucleic acids is placed within the interdiscal space.

EXAMPLE 12

POSTEROLATERAL FUSION

The spine is approached posterolaterially according to known surgical techniques. The composite material of this invention is including osteogenic factor encoding nucleic acids placed between portions of adjacent vertebrae.

EXAMPLE 13

USE OF COMPOSITE WITH BINDING MATRIX

Processed allograft or xenograft chips infused with nucleic acid are added to a binding matrix to hold the chips together, improving their handling characteristics. The chips are added to gelatin and water to form a paste or slurry and then, optionally, freeze dried into a sheet or any other desired form. At the time of surgery the surgeon hydrates the gelatin, graft, nucleic acid composite with a solution, optionally containing nucleic acids encoding osteoinductive factors. Alternatively, the nucleic acid solution could be freeze dried on a hemostatic or other biologically acceptable sponge during manufacture. Alternative binding matrix materials include gelatin, glycosaminoglycans, hyaluronic acid, polymers, proteins and other suitable materials. With or without added demineralized bone matrix, the compositions described herein may have applications in diverse areas of the orthopedic arts. For example, pre-formed shapes may be prepared using appropriately proportioned quantities of bone that has been demineralized, gelatin, growth factors and the like. Compositions wherein gelatin is present at a sufficiently high concentration that the composition is in a semi-liquid, malleable solid or viscous liquid above normal body temperature of a recipient, but becomes a gel or solid at normal body temperature, upon implantation into the recipient are highly desirable. For such applications, gelatin concentrations of between about one to twenty-five percent are typically sufficient, depending on the average molecular weight of the gelatin employed in such compositions. In addition, compositions wherein gelatin is present at a sufficiently high concentration that the composition is a solid at a temperature above normal body temperature of a recipient but is a malleable solid at a slightly higher

temperature, such that a solid of substantially any desired form may be made a the higher temperature, and upon implantation into a recipient, the composition maintains the formed shape, are also highly desirable. Typically, gelatin concentrations of between about ten and forty percent are sufficient for this purpose, depending on the molecular weight of the gelatin employed for such compositions. The nucleic acid encoding growth factors may be added to the binding matrix.

EXAMPLE 14

CHARACTERISTICS AND APPLICATIONS FOR NUCLEIC ACID CONTAINING BONE AND CARTILAGE MATRICES

The combination of a nucleic acid encoding bone growth factor with a bone or cartilage graft provides superior results as compared with other known implant materials. Quicker fusion rates provide enhanced mechanical strength sooner. The graft of this invention is an excellent nucleic acid carrier which provides controlled release of BMP encoding nucleic acids or other osteogenic compositions, including growth factors, cartilage derived morphogenic proteins, nucleic acids encoding BMPs or other growth factors, to the fusion site. The presence of structural collagen and the natural mineral structure of bone results in an elasticity and radioopacity which is identical or nearly identical to bone. The material has sufficient resilience and elasticity to retain a formed body and yet remains rigid enough to maintain an open space between bone portions to result in a fusion mass.

EXAMPLE 15

CARTILAGE AND OTHER TISSUES AS DELIVERY MATRICES FOR NUCLEIC ACIDS

In a manner similar to that used for preparation of nucleic acid impregnated implants, cartilage implants, reduced antigenicity cartilage implants, and other tissues may be produced by treating such tissues with nucleic acids encoding any desirable factor, including but not limited to osteogenic factor, angiogenic factors, and the like. The

antigenicity of such tissues may be reduced by appropriate treatment with chaotropic agents, as described above for bone. The thus-treated cartilage and other tissues are then contacted with various nucleic acids encoding growth factors, cells, proteins, antifungals, antibiotics, antineoplastics, analgesics, and the like, as described above.

While the invention has been illustrated and described in detail in the drawings and foregoing description, the same is to be considered as illustrative and not restrictive in character, it being understood that only the preferred embodiments and best mode have been shown and described and that all changes and modifications that come within the spirit of the invention are desired to be protected.

What is claimed is:

1. An allograft, autograft or xenograft composition comprising a nucleic acid or a mixture of nucleic acids actively encoding one or a plurality of tissue regenerative, osteogenic or chondrogenic growth factors.

2. The composition according to claim 1 wherein said allograft, autograft or xenograft composition is bone or cartilage tissue.

3. The bone or cartilage graft composition of claim 2 wherein said graft has been processed to remove substantially all associated non-collagenous or non-structural collagen proteins, said material containing native collagen materials.

4. The bone or cartilage graft composition of claim 2 wherein said graft has been demineralized or partially demineralized.

5. The bone or cartilage graft composition of claim 2 wherein said graft has not been demineralized.

6. The bone or cartilage graft composition of claim 3 prepared by a process comprising removing associated non-bone adventitious materials from a bone graft to provide a cleaned bone graft, contacting the cleaned bone graft with defatting solutions to provide a cleaned defatted bone graft, and contacting said cleaned defatted bone graft with a chaotropic agent to remove non-collagenous or non-structural collagen proteins.

7. The bone or cartilage graft composition of claim 6 wherein said chaotropic agent is selected from urea, guanidinium hydrochloride, Tween, TritonX-100, and mixtures of these agents.

8. The bone or cartilage graft composition of claim 2 comprising an effective amount to stimulate bone growth of a nucleic acid encoding an osteogenic gene product incorporated within said graft.

9. The bone or cartilage graft composition of claim 2 wherein said bone or cartilage is human, bovine, ovine, equine, porcine, or canine bone, or combinations thereof.

10. The bone or cartilage graft composition of claim 2 machined to form spacers, pins, suture anchors, interference screws, demineralized bone implants, including but not limited to ligaments, oral maxilofacial plates, dowels, posterior lumbar interbody fusion implants, trauma screws and plates, pericardium (for dura, plura, shoulder patch and perioligaments), wedges, chips and pastes comprising reduced antigenicity bone, cartilage or other tissues, alone or in combination with growth factors, or nucleic acids encoding growth factors, including but not limited to bone morphogenetic proteins, cartilage derived morphogenetic proteins, tissue growth factor (beta1 and the like).

11. The bone or cartilage graft composition of claim 10 for maintaining a space between a pair of adjacent vertebrae in a spine, comprising a body sized and shaped to fit within the intervertebral space.

12. The bone or cartilage graft composition of claim 11 prepared by a process comprising removing associated non-bone adventitious materials from a bone graft to provide a cleaned bone graft, contacting the cleaned bone graft with defatting solutions to provide a cleaned defatted bone graft, and contacting said cleaned defatted bone graft with a chaotropic agent to remove non-collagenous or non-structural collagen proteins, wherein said bone graft is either shaped to form a spacer prior to said cleaning, defatting and contacting, or is shaped after said cleaning, defatting and contacting.

13. The bone or cartilage graft composition of claim 12 wherein said graft defines a superior wall for contacting a superior vertebra, an inferior wall for contacting an inferior vertebra and a lateral wall adjacent and between said superior wall and said inferior wall, said lateral wall defining a through hole.

14. The bone or cartilage graft composition of claim 13 wherein said graft is derived from a femoral ring.

15. The bone or cartilage graft composition of claim 13 wherein said graft is derived from a bone dowel.

16. The bone or cartilage graft composition of claim13 wherein said walls define a chamber and said chamber is packed with a pharmaceutically acceptable carrier having a nucleic acid encoding a bone growth factor dispersed therein.

17. The bone or cartilage graft composition of claim 2 wherein said graft has approximately the radioopacity, after implantation, of the bones of the vertebrae between which said spacer is inserted.

18. A composition, comprising: processed bone material composed of bone minerals having a natural crystalline structure of bone and native collagen materials, and an effective amount to stimulate bone growth of a nucleic acid encoding at least one osteogenic factor within said material.

19. An elastic body consisting essentially of structural bone collagen and natural bone minerals in a natural configuration, substantially free of non-collagenous proteins and non-structural collagen protein in combination with an effective amount to stimulate bone growth of a nucleic acid encoding at least one osteogenic factor.

20. A surgical procedure for stabilizing a spine, comprising the steps of: exposing a portion of each of adjacent vertebra requiring stabilization; and placing a

processed bone material within an area between the portions of the adjacent vertebrae, the material composed of bone matrix infused with or coated with a nucleic acid encoding at least one osteogenic factor.

21. The surgical procedure of claim 20 wherein the bone material is formed into an elastic body defining a chamber into which is packed an osteogenic composition in a carrier.

22. The surgical procedure of claim 21 wherein the bone material has dispersed therein said osteogenic factor in a pharmaceutically acceptable carrier.

23. The procedure of claim 20 wherein the portions of the spine are at the posterolateral aspect of the spine.

24. The procedure of claim 20 wherein the material includes bone chips.

25. The procedure according to claim 20 wherein said nucleic acid encodes a bone morphogenetic protein, a cartilage derived growth factor, a tissue growth factor, a bone calcification factor, or a combination thereof.

26. A composition comprising bone or cartilage chips and a nucleic acid encoding a bone morphogenetic protein, a cartilage derived growth factor, a tissue growth factor, a bone calcification factor, or a combination thereof, said composition optionally including proteins, peptides, antineoplastic agents, antiinflammatory agents, antibiotics or combinations thereof.

27. The composition of claim 26 further comprising a binding matrix, said chips disposed within said matrix.

28. The composition of claim 27 wherein said matrix includes gelatin.

29. The composition of claim 28 wherein said gelatin is present at a sufficiently high concentration that said composition is in a semi-liquid, malleable solid or viscous liquid above normal body temperature of a recipient, but becomes a gel or solid at normal body temperature, upon implantation into said recipient.

30. The composition of claim 28 wherein said gelatin is present at a sufficiently high concentration that said composition is a solid at a temperature above normal body temperature of a recipient but is a malleable solid at a slightly higher temperature, such that a solid of substantially any desired form may be made a the higher temperature, and upon implantation into said recipient, the composition maintains said shape.

31. A bone graft composition which is contacted with acid prior to infusion of said graft composition with a nucleic acid.

32. The bone graft composition according to claim 31 wherein said bone graft is contacted with acetic acid, hydrochloric acid, hydrofluoric acid, phosphoric acid, citric acid, formic acid, butyric acid, or mixtures thereof, such that said bone graft is demineralized to an extent between about 0 to 25% of the normal bone mineral content.

33. The bone graft composition according to claim 32 wherein said bone graft has been demineralized to an extent between about 1 to 10% of the normal bone mineral content.

34. The bone graft according to claim 33 wherein said bone graft is demineralized to an extent between about 1 to about 5% of the normal bone mineral content.

35. A method for making a tissue graft for implantation into a recipient in need thereof which comprises:

(a) cleaning a tissue section of unwanted materials;

(b) contacting the thus cleaned tissue section with a nucleic acid, under conditions sufficient to achieve absorption or adsorption of said nucleic acid, wherein said nucleic acid encodes a product the expression of which is desired in the recipient of said tissue graft; and

(c) contacting said nucleic acid contacted tissue section with chemical or energetic agents sufficient to eliminate or inactivate microorganisms, but which do not inactivate said nucleic acid.

36. The method according to claim 35 wherein said tissue is bone.

37. The method according to claim 35, wherein said bone is lyophilized, and then reconstituted in a solution containing said nucleic acid.

38. The method according to claim 35, wherein said bone is soaked in a solution containing said nucleic acid.

39. The method according to claim 36 wherein said bone is further contacted, at any stage prior to the contacting of said bone with said nucleic acid, with sufficient acid for a sufficient amount of time to produce a reduced antigenicity bone containing between with a mineral content that has been reduced by between about 0 to about 25% of the normal bone mineral content.

40. The method according to claim 39 wherein said implant is further contacted with a biologically active agent selected from the group consisting of growth factors, antibiotics, antineoplastics, antifungals, antivirals and combinations thereof under conditions sufficient to permit uptake of said biologically active agent into the matrix of said reduced antigenicity bone.

41. An allograft, autograft or xenograft composition comprising a nucleic acid or a mixture of nucleic acids actively encoding one or a plurality of tissue regenerative, osteogenic or chondrogenic growth factors.

42. The composition according to claim 1 wherein said allograft, autograft or xenograft composition is bone, cartilage, fascia lata, tendon, ligament, peritoneum, dura mater, pericardium, muscle, vasculature, epidermis, dermis or combinations thereof.

43. The composition of claim 41, wherein said composition is in the form of a patch.

44. The composition of claim 41, wherein said growth factors are epidermal growth factor (EGF), transforming growth factor-alpha (TGF-alpha), transforming growth factor-beta (TGF-beta), human endothelial cell growth factor (ECGF), granulocyte macrophage colony stimulating factor (GM-CSF), bone morphogenetic protein (BMP), nerve growth factor (NGF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), insulin-like growth factor (IGF), platelet derived growth factor (PDGF), cartilage derived morphogenetic protein (CDMP), or combinations thereof.

45. The compositions of claim 42, wherein said composition is dermis tissue.

46. The composition of claim 44, wherein said growth factors are VEGF, TGF, ECGF, FGF or combinations thereof.

47. A method of repairing damaged tissue, or stimulating the generation of tissue, comprising the steps of obtaining a section of tissue infused with a nucleic acid or a mixture of nucleic acids actively encoding one or more growth factors, and implanting said section into a patient in need thereof.

48. The method of claim 47 wherein said tissue is vascular tissue, and wherein said implanting comprises joining said section to an artery or vein.

49. The method of claim 47 wherein said one or more growth factors have vascular tissue generating properties.

50. The method of claim 48 wherein said one or more growth factors are VEGF, TGF, ECGF, FGF, or combinations thereof.

51. The method of claim 47 wherein said tissue is in the form of a patch and wherein said patch is adhered to the heart of a patient in need thereof.

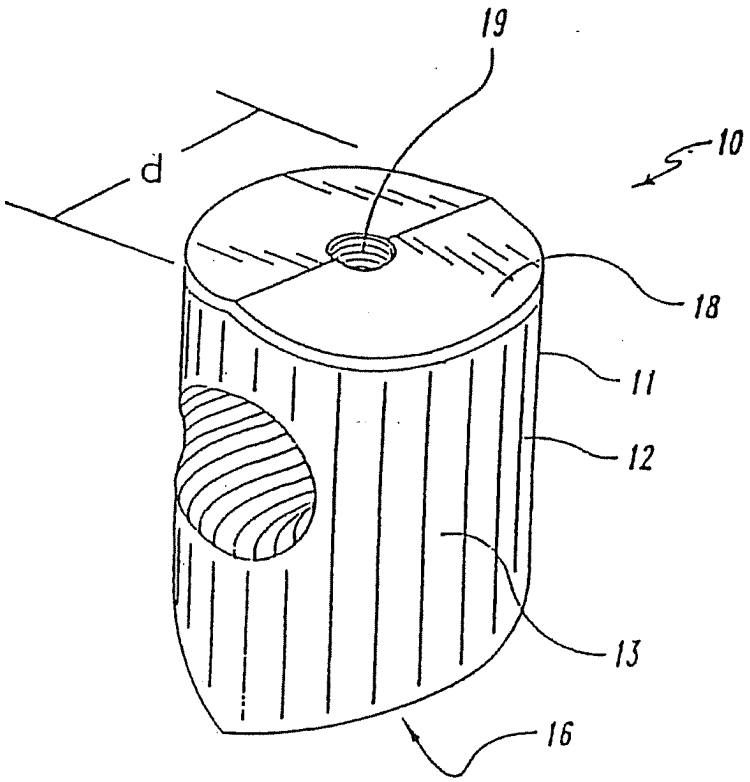

Fig. 1

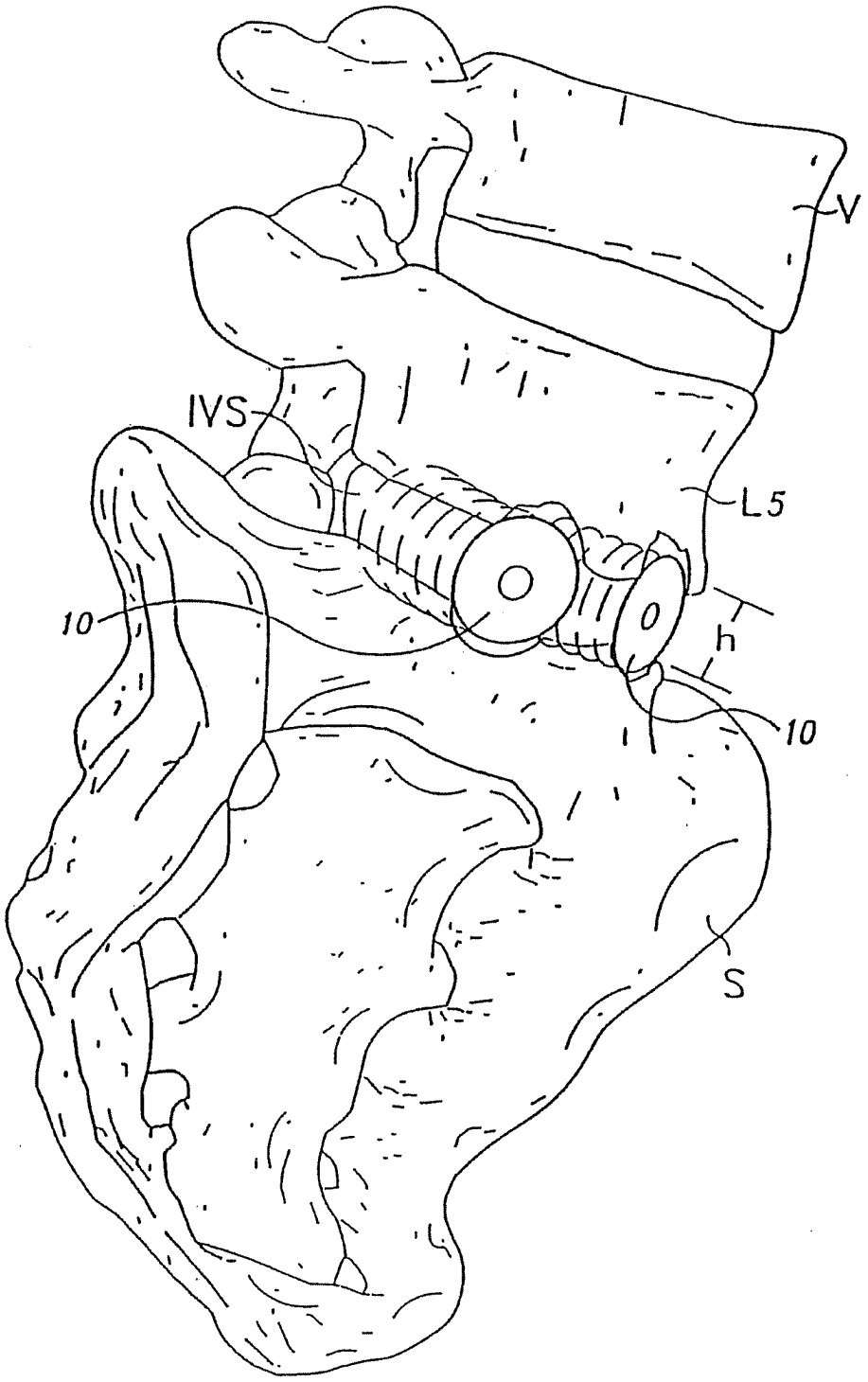

Fig. 2

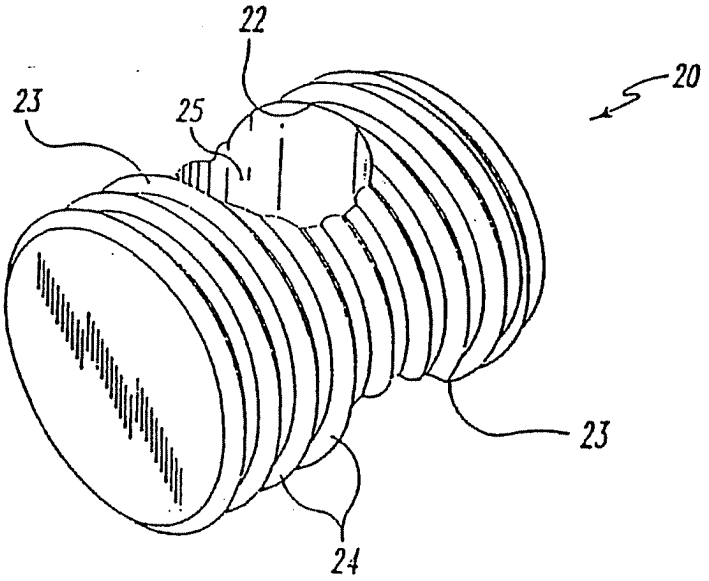

Fig. 3

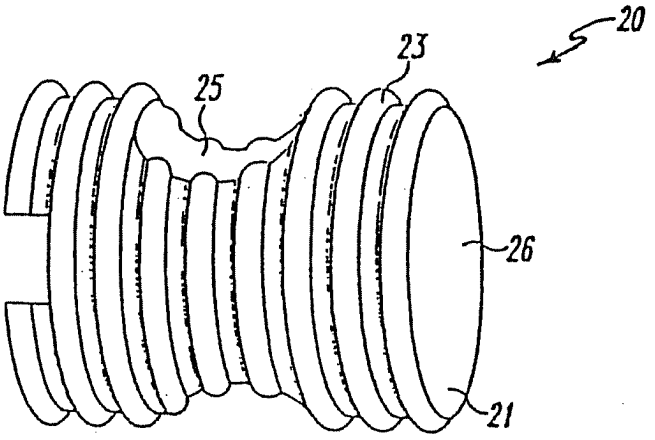

Fig. 4

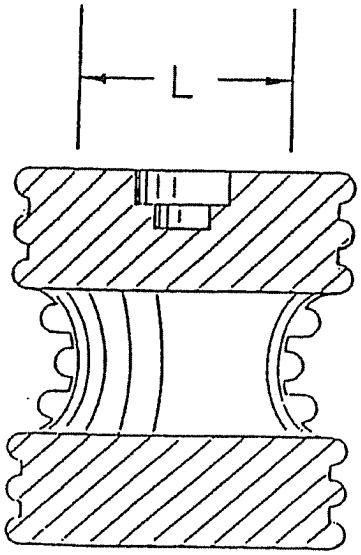

Fig. 5

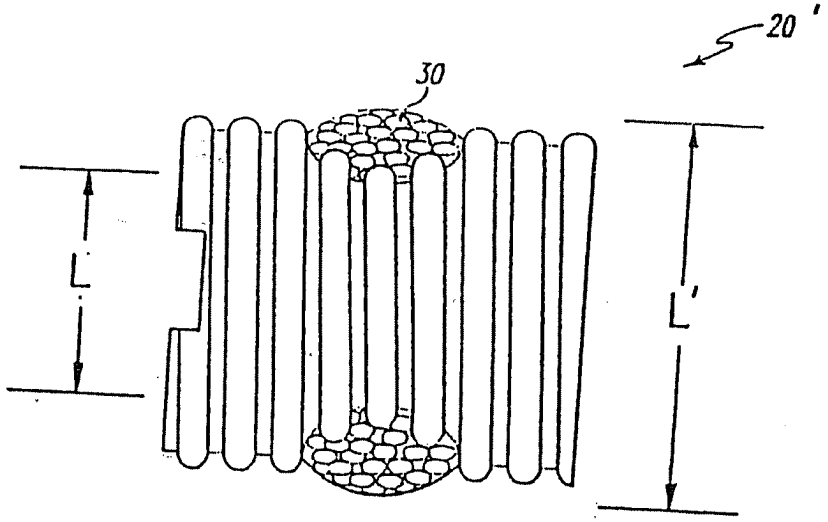

Fig. 6

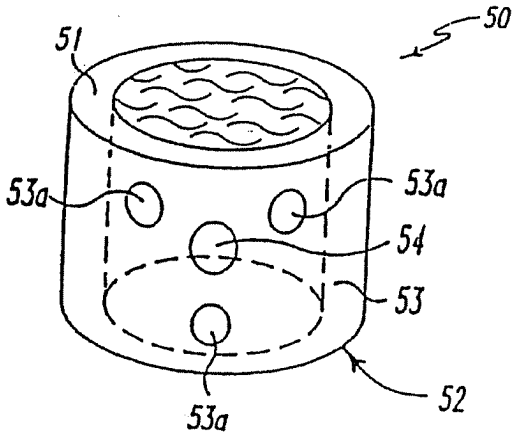

Fig. 7

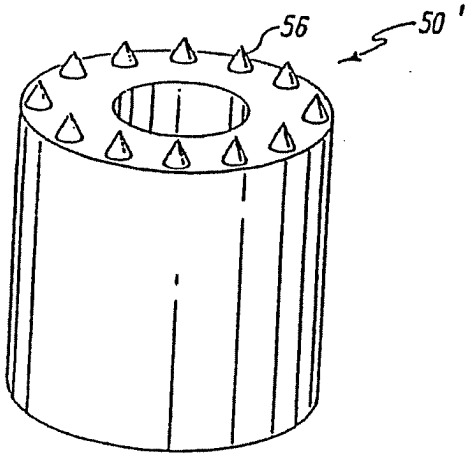

Fig. 8

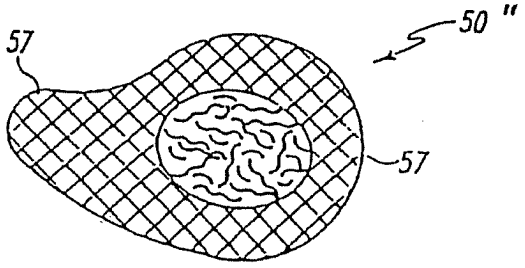

Fig. 9

INTERNATIONAL SEARCH REPORT

Intern ıal Application No
PCT/US 00/20630

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61L27/22 A61L27/36 A61L27/54 C07K14/475 C07K14/51
A61K48/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61L C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 29718 A (GENETICS INST) 17 June 1999 (1999-06-17) page 16, line 5 - line 15; claims; examples	1-51
X	WO 98 12322 A (GENETICS INST) 26 March 1998 (1998-03-26) page 16, line 18 - line 30; claims; examples	1-51
X	WO 99 11664 A (YISSUM RES DEV CO ;GENETICS INST (US)) 11 March 1999 (1999-03-11) page 7, line 14 - line 27; claims; examples	1-51

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
29 November 2000	08/12/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	ESPINOSA, M

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INTERNATIONAL SEARCH REPORT

Intern ıal Application No
PCT/US 00/20630

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 688 678 A (HEWICK RODNEY M ET AL) 18 November 1997 (1997-11-18) column 11, line 1 - line 15; claims; examples	1-51
X	US 5 670 336 A (KUBERASAMPATH THANGAVEL ET AL) 23 September 1997 (1997-09-23) column 31, line 50 - line 55; claims	1-51
X	US 5 763 416 A (BONADIO JEFFREY ET AL) 9 June 1998 (1998-06-09) column 8, line 52 - line 67; claims; examples	1-51
X	US 5 013 649 A (ROSEN VICKI ET AL) 7 May 1991 (1991-05-07) cited in the application claims; examples	1-51
Y	WO 97 38729 A (UNIV MICHIGAN) 23 October 1997 (1997-10-23) cited in the application claims; examples	1-51
Y	WO 98 56433 A (SDGI HOLDINGS INC ;MCKAY WILLIAM F (US)) 17 December 1998 (1998-12-17) cited in the application claims; examples	1-51

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Intern ial Application No
PCT/US 00/20630

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9929718 A	17-06-1999	US 6027917 A	22-02-2000
		AU 1463199 A	28-06-1999
		EP 1037907 A	27-09-2000
WO 9812322 A	26-03-1998	US 5965403 A	12-10-1999
		AU 3655397 A	14-04-1998
		EP 0941323 A	15-09-1999
		FI 990596 A	03-05-1999
		NO 991271 A	16-03-1999
WO 9911664 A	11-03-1999	AU 9306298 A	22-03-1999
		EP 1007559 A	14-06-2000
US 5688678 A	18-11-1997	MX 9203441 A	01-07-1992
		CA 2082941 A	17-11-1991
		EP 0536186 A	14-04-1993
		JP 6500991 T	27-01-1994
		WO 9118098 A	28-11-1991
US 5670336 A	23-09-1997	US 5750651 A	12-05-1998
		US 5258494 A	02-11-1993
		US 5011691 A	30-04-1991
		US 4968590 A	06-11-1990
		US 5814604 A	29-09-1998
		BR 1100734 A	13-06-2000
		US 5958441 A	28-09-1999
		AT 142644 T	15-09-1996
		AT 134647 T	15-03-1996
		AT 124051 T	15-07-1995
		AU 628050 B	10-09-1992
		AU 3444989 A	03-11-1989
		AU 618357 B	19-12-1991
		AU 3530589 A	03-11-1989
		AU 627850 B	03-09-1992
		AU 5174790 A	26-09-1990
		CA 1338663 A	22-10-1996
		DE 68925773 D	04-04-1996
		DE 68925773 T	17-10-1996
		DE 68927153 D	17-10-1996
		DE 68927153 T	06-03-1997
		DE 69020254 D	27-07-1995
		DE 69020254 T	25-01-1996
		DK 411105 T	30-10-1995
		EP 0372031 A	13-06-1990
		EP 0362367 A	11-04-1990
		EP 0411105 A	06-02-1991
		EP 0714665 A	05-06-1996
		EP 0723013 A	24-07-1996
		ES 2074158 T	01-09-1995
		JP 2933867 B	16-08-1999
		JP 8336390 A	24-12-1996
		JP 2869381 B	10-03-1999
		JP 8322570 A	10-12-1996
		JP 8187084 A	23-07-1996
		JP 8024710 B	13-03-1996
		JP 3504736 T	17-10-1991
		JP 2522568 B	07-08-1996
		JP 3500655 T	14-02-1991

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Intern ...al Application No
PCT/US 00/20630

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5670336 A		JP 2548414 B	30-10-1996
		JP 3502579 T	13-06-1991
		WO 8909787 A	19-10-1989
		WO 8909788 A	19-10-1989
		WO 9010018 A	07-09-1990
		US 5468845 A	21-11-1995
		US 4975526 A	04-12-1990
		US 5714589 A	03-02-1998
		US 5863758 A	26-01-1999
		US 5171574 A	15-12-1992
		US 5162114 A	10-11-1992
US 5763416 A	09-06-1998	AT 186327 T	15-11-1999
		AU 698906 B	12-11-1998
		AU 1968695 A	04-09-1995
		CA 2183542 A	24-08-1995
		DE 69513149 D	09-12-1999
		DE 69513149 T	21-06-2000
		DK 741785 T	10-04-2000
		EP 0741785 A	13-11-1996
		ES 2139889 T	16-02-2000
		JP 3054634 B	19-06-2000
		JP 9509825 T	07-10-1997
		WO 9522611 A	24-08-1995
		US 5942496 A	24-08-1999
		US 6074840 A	13-06-2000
		US 5962427 A	05-10-1999
US 5013649 A	07-05-1991	US 5543394 A	06-08-1996
		US 5631142 A	20-05-1997
		US 5459047 A	17-10-1995
		US 5166058 A	24-11-1992
		US 5635373 A	03-06-1997
		US 5849880 A	15-12-1998
		US 5187076 A	16-02-1993
		US 5366875 A	22-11-1994
		US 5939388 A	17-08-1999
		AT 141928 T	15-09-1996
		AU 613314 B	01-08-1991
		AU 7783587 A	29-01-1988
		DE 3751887 D	02-10-1996
		DE 3751887 T	06-03-1997
		DK 53497 A	09-05-1997
		DK 106288 A	28-04-1988
		EP 0313578 A	03-05-1989
		EP 0688869 A	27-12-1995
		ES 2007625 A	01-07-1989
		GR 871028 A	11-01-1988
		IE 75881 B	24-09-1997
		IL 83003 A	31-07-1995
		JP 2729222 B	18-03-1998
		JP 6298800 A	25-10-1994
		JP 3093682 B	03-10-2000
		JP 10070989 A	17-03-1998
		JP 2500241 T	01-02-1990
		JP 2713715 B	16-02-1998
		KR 9705583 B	18-04-1997
		MX 170919 B	22-09-1993

INTERNATIONAL SEARCH REPORT

...ormation on patent family members

Intern... ...al Application No
PCT/US 00/20630

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5013649 A		NZ 220894 A	28-05-1990
		PT 85225 A,B	01-08-1987
		WO 8800205 A	14-01-1988
		US 5618924 A	08-04-1997
		US 5116738 A	26-05-1992
		NO 880701 A	17-02-1988
		NO 963788 A	17-02-1988
		NO 963789 A	17-02-1988
		US 5106748 A	21-04-1992
		US 5141905 A	25-08-1992
		US 5108922 A	28-04-1992
		ZA 8704681 A	27-04-1988
WO 9738729 A	23-10-1997	US 5962427 A	05-10-1999
		AU 710386 B	16-09-1999
		AU 2821297 A	07-11-1997
		CA 2251655 A	23-10-1997
		CN 1226835 A	25-08-1999
		EP 0892644 A	27-01-1999
		NO 984729 A	14-12-1998
WO 9856433 A	17-12-1998	US 5972368 A	26-10-1999
		AU 7818598 A	30-12-1998
		EP 0988070 A	29-03-2000